International Journal of Medicine and Pharmaceutical Science (IJMPS)
ISSN(P): 2250-0049; ISSN(E): 2321-0095
Vol. 6, Issue 1, Feb 2016, 37-46

TRANS STELLAR
•Journal Publications •Research Consultancy

THE ROLE OF ISLET CELL AUTOANTIBODIES, ISLET CELL ANTIGEN-2 ANTIBODY AND ANTIOXIDANT ENZYMES IN DIABETIC PATIENTS TYPE 2

LAMIA MUSTAFA AL-NAAMA[1], JAWAD KADHAM MAHDI[2], NADHAM KADHAM MAHDI[3] & HADI LAZIM AL-ABADY[4]

[1,2,3]*Department of Biochemistry, College of Medicine, University of Basrah, Basrah, Iraq*

[4]*Department of Laboratory Analysis, Technical College of Medical Sciences, Basrah, Iraq*

ABSTRACT

This study included 120 individuals, 60 type 2 diabetic patients and 60 control individuals who had no family history of diabetes mellitus (DM)

Laboratory investigations were done to estimate glutamic acide decarboxilase (GAD) and islet cell antigen-2 (IA-2) autoantibodies by ELISA assay, antioxidant enzymes as glutathion peroxidase (GPX) and superoxide dismutase (SOD) and HbA1c as marker of glycemic control for these patients and control group.

During the period from December 2010 to the end of December 2012. This study enrolled diabetic patients who attended Al-Tahreer General Hospital, Al-Basra Maternity and Pediatric Hospital and Al-Sader Teaching Hospital.

Regarding type 2, it is noticed that 15 (25 %) and 8 (15 %) of those patients were positive for GADA and IA-2A respectively (LADA patients) characterized by certain clinical features that differentiate them from islet cell autoantibodies negatives type 2 diabetics as it was found that the majority of patients with islet cell positivity develop the disease at younger age than those negative patients (57.1% Vs7.7) regarding GADA &(33.3% Vs 2.6%) regarding IA-2A.

Additionally, islet cell autoantibodies positive patients were significantly of higher HbA1c levels than those who were islet cell autoantibodies negative patients (P, 0.001). The difficulty in achieving glycemic control despite OHD and tendency to insulin therapy is attributed to the fact that pathogenesis of diabetes in LADA patients are due to beta cell destruction rather than insulin resistance as in classical type 2 DM.

In addition to that patients of type 2 were significantly lower than control (P < 0.001) in mean activity of both antioxidant enzymes (SOD and GPX) in RBC.

Also lower mean activity of both antioxidant enzymes (SOD and GPX) in RBC were showed higher significant in patients who had uncontrolled diabetes (HbA1c level > 8%) group (P < 0.001).

Regarding LADA group who those showed positive result to GAD and IA-2 autoantibodies showed that significant decrease in mean activity of SOD and GPX in comparison to those negative to autoantibodies of type 2 diabetic patients and also most of them (LADA) had higher HbA1c level > 8%, P< 0.01.

There were significant correlations between the GPX and SOD enzymes with duration of disease and level of HbA_{1c} of type 2 diabetics patients P < 0.01, but there was no significant correlations between the enzymes with Age, P > 0.05.

KEYWORDS: Glutamic Acid Decarboxylase, Glutathione Peroxidase, Islet Cell Antigen-2, Superoxide Dismutase, Type 2 Diabetes

Original Article

Received: Nov 19, 2015; **Accepted:** Dec 23, 2015; **Published:** Jan 20, 2016; **Paper Id.:** IJMPSFEB20165

INTRODUCTION

Type 2 diabetes formerly known as non insulin dependent DM (NIDDM), accounts for essentially the remainder of diagnosed cases of diabetes [(1)] and it results from both decrease insulin secretion as well as the body's inability to effectively utilize the insulin (insulin resistance) [(2,3)].

While the majority of patients fall under the classical definition of either type 1or type 2 diabetes, there are at least two subgroups of patient that bridge these classical barriers, studies indicate that as many as 10-15% of patients diagnosed with type 2 diabetes have circulating autoantibodies to either islet cell antigens and they eventually become insulin dependent [(4)], those patients who are initially misclassified as type 2, are in fact late onset or slow developing type 1 diabetes and some time referred to as latent autoimmune diabetes in adult (LADA) [(5,6)].

Current researches have shown that the measurement of autoantibodies to glutamic acid decarboxylase (GAD), tyrosine phosphetase like protein (IA- 2) and insulin (IAA) can be of a significant value to the clinician in the prediction, diagnosis, and management of patients suffering from diabetes [(7,8)].

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia and the most common complications such as atherosclerosis, nerve damage, renal failure, male impotence and infection [(9)]. Recently, some evidences suggest that oxidative stress may play an important role in the etiology of diabetes and diabetic complications [(10)]. In healthy individuals, oxidative damage to tissue is prevented by a system of defenses which includes antioxidant enzymes and small molecules with scavenging ability such as antioxidant vitamins [(11)]. In diabetic patients an altered balance between reactive oxygen species production and antioxidant levels has been reported [(12,13)] but there is still lack of data regarding the actual status of antioxidant enzymes in diabetic patients.

The aim of the study 9s to estimate autoantibodies GADA and IA-2A as well as the antioxidant enzymes SOD and GPX among diabetic patients type 2.

MATERIALS AND METHODS

Subjects: Sixty diabetic patients type 2 from Basrah Hospitals during the period from November 2010 till December 2012 were included in the study. Further 60 apparantly healthy non-diabetic individuals and they were not first degree relatives for type 2 diabetic patients were considered as control group. Both groups have been instructed and informed about the aim of the study, investigation procedure and their acceptance were taken. The study was ethically approved by the ethical committee of the College of Medicine, University of Basrah, Iraq.

Diagnostic Kits

All the diagnostic kits were purchased from Human, EUROIMMUN from Germany and Randox from United Kingdom these include:

- Anti- GAD ELISA (IgG) Test (Kit No. EA 1022-9601 G) (EUROIMMUN).
- Anti- IA2 ELISA (IgG) Test (Kit No. EA 1023-9601 G) (EUROIMMUN).
- Glycosylated hemoglobin Hb_{A1c} Test(Kit No.10657) (Human).

- Glutathione Peroxidase Test (Kit No. RS 504) (RANDOX).
- Superoxide dismutase Test (Kit No. SD 125) (RANDOX).

All tests were done according to the instruction of the manufacturer.

Laboratory Analysis

After an overnight fasting, 5ml of venous blood was collected from both patient and control subjects, and then divided into the following parts: 2.5ml was transferred to EDTA containing tubes and used for HbA1C estimation within 48 hours. The remaining amount separated by centrifugation at 3000 rpm for 10 min. part of separated plasma is stored in plain tubes at -20^{0}C before testing. Other 2.5ml of whole blood was transferred to heparinized tube and used for glutathione peroxidase and superoxide dismutase enzymes estimation within 48 hours.

Statistical Analysis

Descriptive statistic have been used to describe patient's characteristics by using mean, standard deviation, and percentage (Daniel, 2005). Chi-square test (X2), Pearson Chi-square, Correlation coefficients (r), crosstabs, were used and non diabetic control. Data have been entered and stored in Microsoft Access Software and analyzed by SPSS version 15. The statistically significant differences have been assessed with chi-square test at two levels of probability ($P \leq 0.05$, $P \leq 0.001$).

RESULTS

There were 60 type 2 diabetic patients of both sexes (32 females and 28 males) with age ranged between 34 - 65 years with a mean of 49.6 ± 6.1 years. Duration of disease ranging between 1- 24 years. The control group included 60 (34 females and 26 males) apparantly health non-diabetic subjects and they were not first degree relatives for type 2 diabetic patients. Age ranged 32-62 years with a mean of 47.2± 5.2 years.

The frequency distribution of biochemical parameters among patients was GAD Ab (25%), IA-2 Ab (13.3%). HbA_{1c} (6.7-12.2%), GPX (29.9±2.9 Ug/Hb), SOD (862.8±54.2 Ug/Hb), and Hb g/dl (9-14.2) in comparison to the control group as 0%, 0%, 4.3-5.9%, 49.1±5.2 Ug/Hb, 1249.3±102Ug/Hb respectively (Table 1).

Table 1: Biochemical Characteristic of 6 Study Groups

Bio-Chemical Characteristics	Type 1 DM	Control of Type1 DM	Type 2 DM	Control of Type 2 DM	First Relatives	
					Type 1	Type 2
GAD Ab.	27/40 67.5%	0/40	15/60 25%	0/60	6/40 15%	7/60 11.7%
IA-2 Ab.	16/40 40%	0	8/60 13.3%	0	0	0
%HBA1c	6.7-10.8	4.2 – 5.8	6.7-12.2	4.3 – 5.9	4.1-5.7	4.5-5.7
GPX U/g Hb	31.5±3.4	48.0±3.8	29.9±2.9	49.1±5.2	–	–
SOD U/g Hb	860±60	1282±60.4	862.8±54.2	1249.3±102	–	–
Hb g/dl	9.5-12.7	10.5 -14	9-14.2	11.5-15		

P < 0.01

The distribution of type 2 diabetic patients according to the age of onset were illustrated in (Table 2, 3).

It was shown that those who developed diabetes earlier (before age of 40 years) significantly had higher prevalence of islet cell autoantibodies than those who developed the disease later (after the age of 40), as there were (57.1%) of GADA positive &(33.3%) IA-2A positive type 2 diabetic patients who developed the disease before age of 40 years in comparison to 7.7 % of GADA positive & 2.6 % IA-2A positive type 2 diabetic patients who developed the disease after this age.

Table 2: Distribution of Anti- GAD Ab in Type 2 DM According to the Age of Onset

Results	Positive		Negative		Total
Age of Onset	No.	%	No.	%	
≤ 40year	12	57.1	9	42.9	21
> 40 year	3	7.7	36	92.3	39
Total	**15**		**45**		**60**

X^2 = 17.802 df =1 P < 0.001

Table 3: Distribution of Anti- IA-2 Ab in Type 2 DM According to the Age of Onset

Results	Positive		Negative		Total
Age of onset	No.	%	No.	%	
≤ 40year	7	33.3	14	66.7	21
> 40 year	1	2.6	38	97.4	39
Total	**8**		**52**		**60**

X^2 = 11.183 df =1 P < 0.001

It was shown from the present study that the prevalence of islet cell autoantibodies is affected by the duration of the disease, (Table 4,5) as the percentage of autoantibodies in patients with duration of disease less than 5 years were 14.3 % & 9.5 % for GADA & IA-2A respectively and the percentage begin to change to 42.9%, 0 for GADA & IA-2A respectively in those with duration of disease more than 13 years duration.

The relation of GADA and IA-2A with the duration of disease showed no significant differences (P > 0.05) (Table 4, 5).

Table 4: Influence of Duration of the Disease in the Prevalence of GADA in Type 2 Diabetes

Result	Positive		Negative		Total
Duration	No.	%	No.	%	
< 5	3	14.3	18	85.7	21
5 -8	6	33.3	12	66.7	18
9 -12	3	21.4	11	78.6	14
> 13	3	42.9	4	57.1	5
Total	**15**		**45**		**60**

X^2= 5.981 df= 4 p > 0.05

Table 5: Influence of Duration of the Disease in the Prevalence of IA-2 A in Type 2 Diabetes

Result	Positive		Negative		Total
Duration	No.	%	No.	%	
< 5	2	9.5	19	90.5	21
5 -8	3	16.7	15	83.3	18
9 -12	3	21.4	11	78.6	14
> 13	0	0	7	100	7
Total	**8**		**52**		**60**

X^2= 5.981 df= 4 p > 0.05

According to HBA1c levels, the diabetic patients were divided into three groups, patients with good diabetic control (GDC) with HBA1c level less than 7.0%, patients with fair diabetic control (FDC) with HBA1c level between 7.0%- 8.0% and patients with poor diabetic control (PDC) with HBA1c level more than 8.0% (Table 6).

As expected, there are significant differences in mean level of HBA1c among three groups of both types of patients ($p < 0.01$ in all cases).

Table 6: Frequency Distribution of Patients of Both Types and Control. According to HBA1c Levels

Groups	Type 1 Diabetes			Type 2 Diabetes		
Sub-Groups	**No.**	**%**	**HbA_{1c} Mean ± SD**	**No.**	**%**	**HbA_{1c} Mean ± SD**
G DC Good diabetic control < 7%	6	15	6.8 ± 0.08	8	13.3	6.83 ± 0.07
F D C(Fair diabetic control) (7% – 8%)	8	20	7.7 ± 0.15	9	15	7.6 ± 0.18
P D C (Poor diabetic control) > 8%	26	65	9.3 ± 0.68	43	71.7	9.6 ± 1
Total	**40**	**100**	**8.6 ± 1.1**	**60**	**100**	**8.9 ± 1.4**

The activities of SOD and GPX in patients and control were determined. As shown in (Table 7), significant reduction in the activities of both enzymes in both types of patients was noticed ($p<0.001$).

Table 7: Mean Activity of SOD and GPX in Patients and Control

Variables	Type 1 Diabetes		T	P	Type 2 Diabetes		T	P
	Patients	Control			Patients	Control		
GPX (U/gHb)	31.5± 3.38	48.0±3.8	-20.4	.00	29.8±3.1	49.1±5.1	-24.6	.00
SOD (U/gHb)	860±60	1282±60.4	-31.3	.00	862±55.1	1249±102	-25.9	.00

Data are presented as mean ± SD. P < 0.001
T= -24.6
P < 0.001

In type 2 diabetic patients possible correlation between the activities of antioxidant enzymes, SOD and GPX with age, duration of diabetes and levels of HbA_{1c} were also studied. As showed in (Table 8). There were significant correlations between the GPX and SOD enzymes with duration of disease and level of HbA_{1c} of type 2 diabetics patients $P < 0.01$, but there was no significant correlations between the enzymes with Age, $P > 0.05$.

Table 8: Correlation between Glutathione Peroxidase (Gpx), Superoxide Dismutase (SOD) and Clinical Characteristics of Type 2 D.M Patients

Clinical and Biochemical Characteristics	Type 2 D.M GPX (U/g Hb)		Type 2 D.M SOD (U/g Hb)	
	r	P	R	P
Age (year)	- .142	> 0.05	- .004	> 0.05
Duration of diabetes (year)	- 0.358**	< 0.05	-0.0311*	< 0.05
HbA1c %	- 0.619**	< 0.05	- 0.551**	< 0.05
Clinical and Biochemical Characteristics	**Control GPX (U/g Hb)**		**Control SOD (U/g Hb)**	
	r	**P**	**R**	**P**
Age (year)	**- .521****	**P < 0.05**	**- .537****	**P < 0.05**

****Correlation is significant at the 0.01 level.**
*** Correlation is significant at the 0.05 level.**

DISCUSSIONS

Autoimmune diabetes is subdivided into a rapidly progressive form that is commonly seen in children and early adulthood and more slowly progressive form of type 1 diabetes was termed latent autoimmune diabetes of adults (LADA; referring to the latency of autoimmunity) or type 1.5 diabetes. However, the late age of clinical presentation (with onset after the age of 30 years) and absence of immediate insulin requirement they always misdiagnosed as type 2 diabetes [14,15].

The diagnosis of LADA is difficult due to a lack of defining features; most authors propose that LADA should have three features including: adult age at diagnosis, the presence of diabetes-associated autoantibodies, and delay from diagnosis in the need for insulin therapy to manage hyperglycemia [16]. However, the first and last are not categorical traits, being dependent on the mode of ascertainment and decision making by physicians. The second feature lacks disease specificity because it is based on positivity for autoantibodies found in type 1 diabetes mellitus.

In an attempt to identify the prevalence of LADA in those who are clinically diagnosed as type 2 DM, a 60 type 2 diabetic patients were investigated for the presence of islet cell autoantibodies (GADA&IA-2A), as they considered as markers for autoimmunity, and correlate their presence with some clinical variables to establish the diagnosis of LADA and to predict insulin dependency in those patients. From 60 type 2 diabetic patients, there were 15(25 %) GADA positive, 8(13.3%) IA-2A positive, and the percentage of these antibodies increased up to 28.3 % when GADA & / IA-2A positive results calculated together.

This prevalence in patients phenotypically diagnosed as type 2 diabetes is in agreement with prevalence of LADA in patients reported by Turner [17] in United Kingdom Prospective Diabetic Study (UKPDS) as they registered a 25-34% of LADA patients in type 2 diabetics, also this finding is in agreement with 0ther [18], as they demonstrated the relative high frequency of this form of diabetes (approximately 20%,) among type 2 diabetic patients in the age range 25-44 years. Also agreement with prevalence of LADA in patients reported by Torn et al. demonstrated that that 47% of type 2 and 59% of unclassifiable patients at diagnosis in the age range from 15 to 34 years had B- cell autoimmune antibodies in Sweden [19]. Humphrey et al. demonstrated that 37% of adult onset insulin treated patients had positive GADA levels on a population based study in Australia [13]. Jasem, el al demonstrated that 18.9% of adults on oral hypoglycemic treated patients had positive GADA levels in Iraq[20].

In contrast, Lutale et al. found low prevalence of GADA and IA-2A autoantibodies (7.3%) among the young onset diabetes subjects of African origin in Dar elSalaam, Tanzania. concluding low prevalence of LADA among these patients [21]. Park et al. also revealed low prevalence of GADA of 1.7% in newly diagnosed type 2 Korean patients age over 30 years from a population based study [22] and so do Thai et al. [23]. The prevalence of LADA in Italy was estimated in a population based study and it was 2% of all cases of adult diabetes and 2.8% of those diagnosed [24].

This result is in agreement with other studies [,25,26.27.) who estimated a significantly higher prevalence of these antibodies (GADA, IA-2A) in young patients with type 2 DM. The explanation of this finding may be due to the facts that those patients who develop the disease before age of 40 years have HLA-DR3/DR4 and show significant higher frequencies of anti-islet cell antibodies while those who begin the disease after 40 years were HLADRB1/DRQB1 which show much less evidence of autoimmunity [28,29,30].

When the duration of the disease is taken in consideration as a factor affecting the frequency of these antibodies, it was found that the frequency of these antibodies not significant relations with the duration of the disease.

These results were controversial, when discussed, as many studies showed the same results like [20], and who are demonstrated a increase of antibodies with time [21] and who are showed other studies decrease and disappearance of these autoantibodies with time[31,32] and they explained this finding by seroconversion of patients from GADA & IA- 2A negative at onset of disease to GADA & IA-2A positive later on. These differences may vary greatly with race and antibody assay methods [33].

This finding is in agreement with a number of studies [34,35.36,37,38,39,40] and are not compatible with others [4142,43,44,45,46,47,].

In diabetic patients, the autoxidation of glucose results in the formation of hydrogen peroxide which inactivates SOD [48]. Therefore, the accumulation of H_2O_2 may be one of the explanations for decreased SOD in these patients. Also, the characteristic feature of diabetes, hyperglycemia, enhances non-enzymatic binding of glucose to proteins. This phenomenon, glycation, causes structural and functional changes in the proteins like hemoglobin, albumin, basal membranes of glomeruli, etc.

Antioxidant enzymes are endogenous proteins that work in combination to protect cells from reactive oxygen species (ROS) damage. Increased levels of the products of oxidative damage to lipids and protein have been detected in the serum of diabetic patients and their presence correlates with the development of complications [49].

Similar findings were reported by various other studies[46,50,51,52,53,54,55]. The diabetic patients had shown an antioxidant activities of enzymes such as SOD and GPx were markedly diminished in comparison to controls.

However, some authors found no differences between GPX activity of types I or II diabetic patients and control [54,22].

Thus showing that the presence of circulating autoantibodies could in some way contribute to the poor action of insulin in a percentage of patients with type II diabetes mellitus. Thus autoantibodies could be associated with increased insulin resistance. This fact agree with other study[56].

There is significantly higher prevalence of GAD and IA-2 autoantibodies of islet cell among patients with type 2 diabetes in comparison to the non diabetic control group. Oxidative stress SOD and GPX are depleted among diabetic patients. In addition, there is negative correlation between SOD and GPX depletion and poor diabetic control that reflect the more oxidative stress with poor diabetic patients may progress complications.

REFERENCES

1. *Defronzo, R. A. (1997). Pathogenesis of type 2 Diabetes. Rev.5:177.*
2. *Forte, D. JR. (1991). Banting Lecture 1990: B-cells in type 2 DM. Diabetes. 40: 166 – 180.*
3. *Accilli, D. (2004). The struggle for mastery in Insulin Action. From triumvirate to republic diabetes. Diabetes. 53: 1633-1642.*
4. *Humphrey, A. R. G.; Mc Carty, I. R.; Rowely, MJ. Dwyer T, Zimmet P; (1998). autoantibodies to GAD and phenotypic feature associated with early insulin treatment in adults with adult – onset diabetes mellitus. Diabetic Med. 15 :113 – 119.*
5. *Seissler, J.; De Somaville, J. J.; Morgenthaler, N. G.; Steinbrenner H, Glawe D, Khoo-Morgenthaler UY, et al (1998). immunological hetrogencity in type 1 diabetes: presence of distinct autoantibody patterns in patients with acute onset and slowly progressive disease. Dialectologia.41:891-897.*

6. *Naik, R. G. and Palmer, J. P. (2003). Latent autoimmune diabetes in adult (LADA). Rev. Endoc. Metab. Disord. 4: 233-241.*
7. *Palmer, J. P. and Mcculloch, D. K. (1991). prediction and prevention of IDDM. diabetes.40 : 943-947.*
8. *Kawasaki, E. ; Abiru, N. and Eguchi, K. (2004). Prevention of type 1 diabetes : from the view point of beta cell damage. Diabetes Res. Clin. Pract. 66 (1) : 27-32.*
9. *Bennett P : Definition, diagnosis and classification of diabetes mellitus and impared glucose tolerance. In:Joshin's diabetes mellitus. Ed by C Kahn and G Weir, 13th ed, Lea and Febiger Co, USA, pp 193-200, 1994.*
10. *Shinn SH : Oxidative stress and diabetic vascular complications. In: Recent advances on pathogenesis and management of diabetes mellitus. 1st ed, Elsevier Science Co, Singapore, pp 3-8, 1998.*
11. *Polidori MC, Mecocci P, Stahl W, Parente B, Cecchetti R, Cherubini A., et al (2000): Plasma levels of lipophilicantioxidants in very old patients with type II diabetes. Diabetes Metab, Res Rev, 16:15-19.*
12. *Nourooz-Zadeh J, Rahimi A, Tajaddini-Sarmadi J, Tritschler3, P. Rosen4, B. Halliwell5, D. J. Betteridge1 et al. (1997) : Relationship between plasma measures of oxidative stress and metabolic control in NIDDM. Diabetologia, 40:647-653.*
13. *Sšzmen Ey, Sšzmen B, Delen Y and Onat T(2001) : Catalase/Superoxide Dismutase (SOD) and Catalase/Paraoxanase (PON) ratio may implicate poor glycemic control. Archives of Medical Research, 32:283-287.*
14. *Pihoker, P. (2006). What type of diabetes do young people have? Current Diabetes Reports. 6(2): 108.*
15. *Notkins AL, Lernmark A(2001). Autoimmune type 1 diabetes: resolved and unresolved issues. J Clin Invest, 108:1247–1252.*
16. *David D, Leslie G, Williams R, Pozzilli P. Leslie RD[1], Williams R, Pozzilli P. (2006) : Type 1 Diabetes and Latent Autoimmune Diabetes in Adults: One End of the Rainbow The Journal of Clinical Endocrinology and Metabolism, 91(5):1654-1659.*
17. *Turner R, Stratton I, Horton V, Turner R, Stratton I, Horton V et at (1997). UKPDS 25: autoantibodies to islet cell cytoplasm and glutamic acid decarboxylase for prediction of insulin requirement in type 2 diabetes. Lancet 350: 1288-1293.*
18. *Seissler, J. and Scherbaum, W. A.(2006). Autoimmune diagnostic in diabetes mellitus. Clin. Chem. Lab. Med. 44(2): 133-137*
19. *Torn C, Landin-Olsson M, Ostman J(2000): Glutamic acid decarboxylase antibodies (GADA) is the most important factor for prediction of insulin therapy within 3 years in young adult diabetic patients not classified as Type 1 diabetes on clinical grounds. Diabetes Metab Res Rev, 16:442-447.*
20. *Jasem M A. AL-Ubaidi A A. Admon A. Zwaer KN (2010). Prevalence of (LADA) among clinically diagnosed type 2 diabetic patients Medical Journal of Islamic World Academy of Sciences; 18:2, 49-54).*
21. *Lutale, J. J. K. ; Thordarson, H. ; Holm, P. I. ; GE Eide **;** K Vetvik. (2007). Islet cell autoantibodies in African patients with type 1& type 2 diabetes in Dar el Salaam Tanzania. Journal of autoimmune disease. 4: 45-53.*
22. *Park Y, Lee HK, Koh CS, Min H, Rowley M, Mackay IR, et al (1996) : The low prevalence of immunogenetic markers in Korean adultonset IDDM patients. Diabetes Care, 19:241-245.*
23. *Thai AC, Ng WY, Loke KY, Lee WRW, Lui KF, Cheah JS., (1997). Anti-GAD antibodies in Chinese patients with youth and adult-onset IDDM and NIDDM. Diabetologia, 40:1425-1430.*
24. *Maioli M, Alejandro E, Tonolo G, Gilliam LK, Bekris L, Hampe CS, et al (2004): Epitope-Restricted 65-Kilodalton Glutamic Acid Decarboxylase Autoantibodies among New-Onset Sardinian Type 2 Diabetes Patients Define Phenotypes of Autoimmune Diabetes The Journal of Clinical Endocrinology and Metabolism, 89(11):5675-5682.*

25. *Lohmann, T.; Kellner, K.; Verlohren, H-J.; Krug J, Steindorf J, Scherbaum WA, Seissler J., (2001). Titre and combination of ICA and autoantibodies to glutamic acid decarboxylase discriminate two clinically distinct types of latent autoimmune diabetes in adults (LADA). Diabetologia. 44: 1005–1010.*

26. *Elbein, S. C.; Wegner, K. and Miles, C. (1997). The role of late onset autoimmune diabetes in white familial NIDDM. Diabetic Care. 2018:1248-1251.*

27. *Nabhan, F.; Emanuele, M. A. and Emanuele, N.(2005). Latent autoimmune diabetes of adulthood : unique features that distinguish it from type 1 &2. Post grad. Med. 117(3): 7-12.*

28. *Groop, L.; Miettinen, A.;Groop, P. H. M.; Meri S, Koskimies S, Bottazzo GF.(1988). Organ specific autoimmumity and HLA-DR antigens as marker for beta cell destruction in patients with type 2 diabetes. diabetes. 37: 99-103.*

29. *Pozzilli, P. and Di Mario, U.(2001). Autoimmune diabetes not requiring insulin at diagnosis (LADA): definition, characterization and potential prevention. Diabetes Care. 24 (8):1460-1467.*

30. *Hosszufalusi, N. ; Vatay, A. and Rajczy, K. (2003). Similar genetic features and different islet cell aut30- Hosszufalusi, N. ; Vatay, A. and Rajczy, K. (2003). Similar genetic features and different islet cell autoantibody pattern of latent autoimmune diabetes in adult (LADA) compared with adult onset type 1 diabetes with rapid progression. Diabetic care. 26: 452-457.*

31. *Pardoni, VC.;Mourao, DM.: Nascimento, PD.; Vívolo MA, Ferreira SR, Pardini H. (1999). frequency of islet cell autoantibodies (IA-2A, GAD) in young Brazilian type 1 diabetic patients. Brazilian Journal of Medical & Biological research. 32(10): 1195-1198.*

32. *Deblock, CEM. ; Deleeuw, IH. ; Vertommen, JJF. ; Rooman RP, Du Caju MV, Van Campenhout CM (2001). Beta cell, thyroid, gastric, adrenal & celiac autoimmunity & HLA-DQ types in type 1 diabetes. Clin. Exp. Immunol. 126 (2): 236- 241.*

33. *Damanhouri LH, Dromey JA, Christie MR, Nasrat HA, Ardawi MS, Robins RA, Todd I. (2005). Autoantibodies to GAD and IA-2 in Saudi Arabian diabetic patients; Diabet. Med. 22, 448-452.*

34. *Hisalkar. PJ, Patne1 AB, Fawade MM, Karnik AC,(2012). Evaluation of plasma superoxide dismutase and glutathione peroxidase in type 2 diabetic patients. Biology and medicine 4(2):65-72.*

35. *Rema M, Mohan V, Bhaskar, AShanmugasundaram KR, et al. (1995). Does oxidant stress play a role in diabetic retinopathy?. Indian J Ophthalmol 43:17-21*

36. *Hartnett E M, Stratton R D, Browne R W, Rosner BA, Lanham RJ, Armstrong D et al [1]. (2000). Serum Markers of oxidative stress and severity of diabetic retinopathy;Diabet. Care**23** 234–240.*

37. *Kesavulu MM, Kameswararao B, Apparao Ch, Kesavulu MM[1], Kumar EG, Harinarayan CV. et al (2002). Effect of ω-3 fatty acids on lipid peroxidation and antioxidant enzyme status in type 2 diabetic patients. Diabetes Metab. 28:20-26.*

38. *Bhatia, S., M. S. Venkata, G. J. Kaur, P. K. Gambhir J, Madhava Prabhu K., (2003). Antioxidant status, lipid peroxidation and nitric oxide end products in patients of type 2 diabetes mellitus with nephropathy. Clin. Biochem., 36: 557-562.*

39. *Indran M, Rokiah P, Chan SP, Kuppusamy UR (2004) : Alteration of Lipid Peroxidation and Antioxidant Enzymes in Young Malaysian IDDM Patients. Med J Malaysia No 2 June. Vol59 : 166-170.*

40. *Abou-Seif A, Youssef A (2004). Evaluation of some biochemical changes in diabetic patients. Clin. Chim. Acta 346:161-170.*

41. *Kaji H, Kurasaki M, Ito K, Saito T, Saito K, Niioka T, et al. (1985) Increased lipoperoxide value and glutathione peroxidase activity in blood plasma of type Í diabetic women. Klin Wochenschr 63:76576–76578.*

42. *Bono A, Caimi G, Catania A, Sarno A, Pandolfo L (1987) Red cell peroxide metabolism in diabetes mellitus. Horm Metab Res 19:264–266.*

43. *Godin DV, Wohaieb SA, Garnett ME, Goumeniouk AD., (1988) Antioxidant enzyme alterations in experimental and clinical diabetes. Mol Cell Biochem 84:223–231*

44. *Telci A, Cakatay U, Salman S, Satman I, Sivas A. (2000). Oxidative protein damage in early stage Type 1 diabetic patients. Diabetes Res. Clin. Pract., 50:213–223.*

45. *Palanduz S, Ademoğlu E, Gökkuşu C, Tamer S. (2001). Plasma antioxidants and type 2 diabetes mellitus. Palanduz S, Ademoğlu E, Gökkuşu C, Tamer S. Res Commun Mol Pathol Pharmacol. 109(5-6):309-18.*

46. *Turk HM, Sevinc A, Camci C, Cigli A, Buyukberber S, Savli H, Bayraktar N. (2002). Plasma lipid peroxidation products and antioxidant enzyme activities in patients with type 2 DM. Acta Diabtologica, 39(3): 117-122.*

47. *Memisoğullari R, Taysi S, Bakan E, Capoglu I. (2003). Antioxidant status and lipid peroxidation in type 2 diabetes mellitus. Cell Biochem. Funct., 21: 291-296.*

48. *Fajans S(1995) : Diabetes mellitus, definition, classification, tests. In: Endocrinology Degroat L, 3rd ed, Saunders Co, USA, pp 1411-1422.*

49. *Brownlee M et al, (2001). Biochemistry and molecular cell biology of diabetic complications. Nature, 414:813-820. Available from www.Nature.com.*

50. *Mahboob M, Rahman MF, Grover P,(2005). Serum lipid peroxidation and antioxidant enzyme level in male and female diabetic patients. Singapore Medical Journal, 46(7): 322–324.*

51. *Targher G, Bertolini L, Zoppini G, Zenari L, Falezza G. (2005). Increased plasma markers of inflammation and endothelial dysfunction and their association with microvascular complications in type 1 diabetic patients without clinically manifest macroangiopathy. Diabetic Medicine, 22(8): 999–1004.*

52. *Singhania N, Puri D, Madhu SV, Singhania N[1], Puri D, Madhu SV et al, (2008). Assessment of oxidative stress and endothelial dysfunction in Asian Indians with type 2 DM with and without macroangiopathy. QJM, 101: 449–455.*

53. *Sailaja YR, Beskar R, Saralakumari D,(2003). The antioxidant status during maturation of reticulocytes to erythrocytes in type 2 diabetics. Free Radical Biology & Medicine, 35: 133–139.*

54. *Takeda H, Kawasaki E, Shimizu I, Konoue E, Fujiyama M, Murao S, et al (2002). Clinical, autoimmune, and genetic characteristics of adult-onset diabetic patients with GAD autoantibodies in Japan (Ehime Study). Diabetes Care, 25:995-1001.*

55. *Rahimi-Pour, A, RahbaniI-Nobar ME. AdiI-Beig F MIrhashemi SM. (1999) Total antioxidants capacity, SOD and GPX in diabetic patients ; Medical Journal of Islamic Academy of Sciences 12:4, 109-114.*

56. *Panczel P Hosszufalusi N, Bornemisza B, Illyés G, Halmos T, Baranyi E et al. (1999): "Detection of antibodies against pancreatic islet cells in clinical practice." III. Belgyogyaszati, Klinika, Semmelweis Orvostudomanyi Egyetem, Budapest. Orv Hetil. Nov 28; 140(48):2695-701*